



Review

# Cytokinin-Controlled Gradient Distribution of Auxin in *Arabidopsis* Root Tip

Lei Wu , Jun-Li Wang, Xiao-Feng Li and Guang-Qin Guo \*

Institute of Cell Biology and MOE Key Laboratory of Cell Activities and Stress Adaptations, School of Life Sciences, Lanzhou University, Lanzhou 730000, China; leiwu@lzu.edu.cn (L.W.); wangjl19@lzu.edu.cn (J.-L.W.); xfli@lzu.edu.cn (X.-F.L.)

\* Correspondence: gqguo@lzu.edu.cn

**Abstract:** The plant root is a dynamic system, which is able to respond promptly to external environmental stimuli by constantly adjusting its growth and development. A key component regulating this growth and development is the finely tuned cross-talk between the auxin and cytokinin phytohormones. The gradient distribution of auxin is not only important for the growth and development of roots, but also for root growth in various response. Recent studies have shed light on the molecular mechanisms of cytokinin-mediated regulation of local auxin biosynthesis/metabolism and redistribution in establishing active auxin gradients, resulting in cell division and differentiation in primary root tips. In this review, we focus our attention on the molecular mechanisms underlying the cytokinin-controlled auxin gradient in root tips.

**Keywords:** cytokinin; auxin; biosynthesis/metabolism; auxin transport; cell division; cell differentiation; root meristem



**Citation:** Wu, L.; Wang, J.-L.; Li, X.-F.; Guo, G.-Q. Cytokinin-Controlled Gradient Distribution of Auxin in *Arabidopsis* Root Tip. *Int. J. Mol. Sci.* **2021**, *22*, 3874. <https://doi.org/10.3390/ijms22083874>

Academic Editor: Takeshi Nishimura

Received: 19 March 2021

Accepted: 6 April 2021

Published: 8 April 2021

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Roots are plant organs, usually located below the surface of the soil, where they grow and respond to various environmental stimuli. Plants receive essential water and nutrients from the soil through roots. Thus, overall plant survival depends on root growth and development. Auxin is the most important phytohormone that regulates the growth and development of plant roots [1–5]. Although auxin plays a central role in the control of root growth and development, much evidences have now been collected showing that the correct growth and development of roots also depends on auxin cross-talk with other phytohormones, such as cytokinin [6,7]. Since the discovery of cytokinin as an inducer of cell division in cultured plants, the function of cytokinin has been linked to that of auxin [8,9]. Earlier studies have suggested an antagonistic relationship between these two phytohormones; however, the truth is more complicated, with both antagonistic and supportive interactions that are usually cell and/or tissue-specific [10–12].

It is usually considered that the key to the function of auxin in plant growth and development is its gradient distribution [13,14]. Of all the mechanisms that regulate auxin distribution, cytokinin plays a prominent role, not only by regulating local auxin metabolism [15–22], but also by modulating PAT (polar auxin transport) [11,23–27]. In the last decade, multiple points of cross-talk between auxin and cytokinin, including biosynthesis/metabolism, transport, and signaling, have been revealed [12,23,28–48]. In this review, we focus on cytokinin-controlled gradient distribution of auxin by regulating its biosynthesis and transport, and its role in regulating root growth and development.

## 2. Cytokinin Signal Pathway

The cytokinin signaling pathway in plants is similar to the bacterial multi-step two-component signal transduction system [12,34,36,49]. In *Arabidopsis*, cytokinin binding leads to autophosphorylation of membrane-bound cytokinin receptors AHK2 (*Arabidopsis*

histidine kinase 2), AHK3 and AHK4/CRE1 (cytokinin response 1), followed by a phosphorylation cascade [50–53]. The phosphoryl group is transferred from receptors to AHPs (Arabidopsis histidine phosphotransferase proteins) [54–57], which enters the nucleus and phosphorylates the ARR (Arabidopsis response regulators). ARRs can be divided into two types according to their structure. Phosphorylated type-B ARRs work as TFs (transcription factors), activating cytokinin-responsive genes [58–61]. Unlike the type-B ARRs, the type-A ARRs lack a DNA-binding domain, and their expression is rapidly induced by cytokinin, which forms a feedback loop by negatively regulating type-B ARRs [62–65]. Furthermore, some CRFs (cytokinin responsive factors), identified as AP2 TFs [66,67], also play a role in cytokinin-regulated gene expression [67].

### 3. Cytokinin-Regulated IAA Biosynthesis

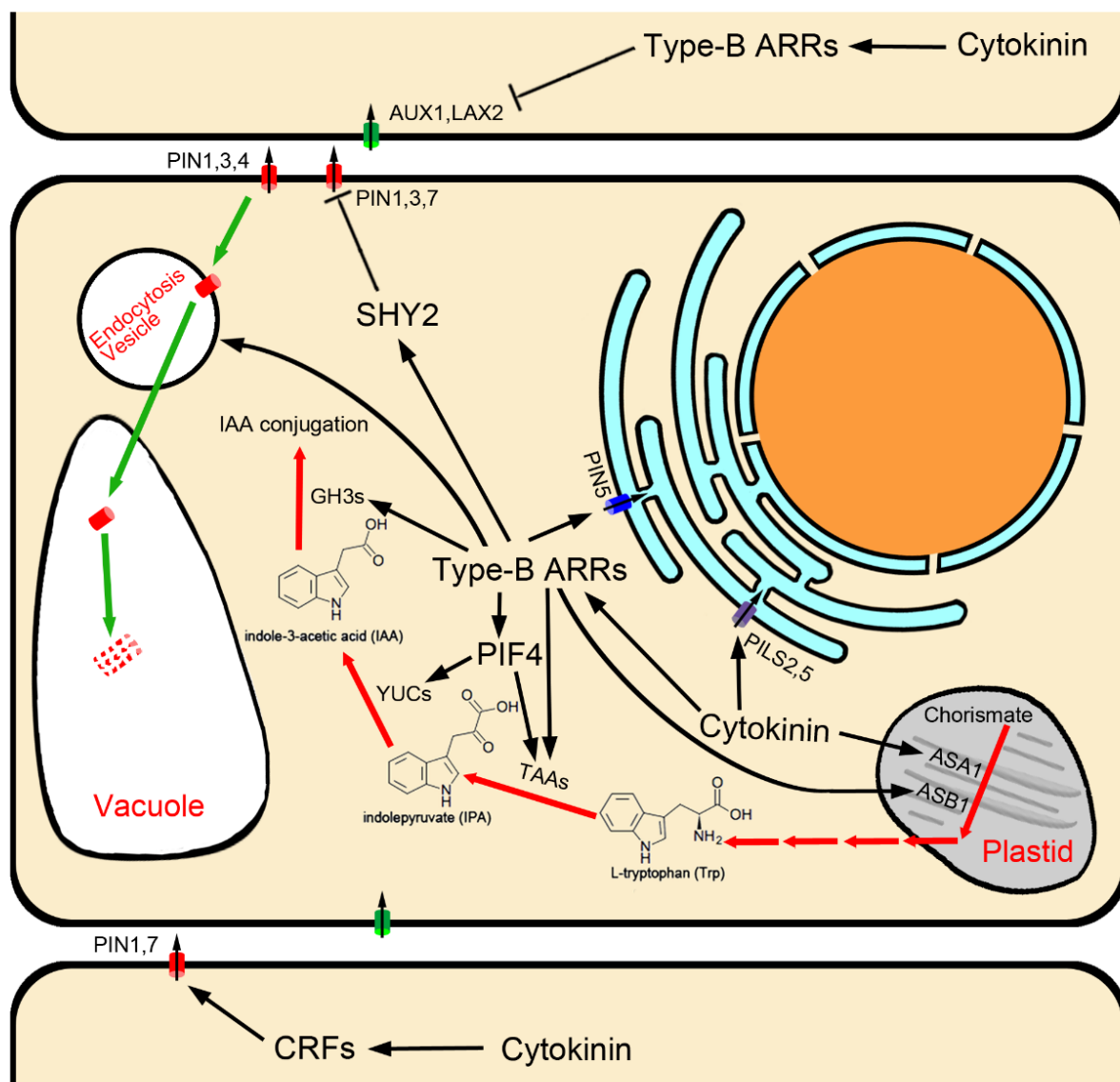
Based on biochemical and genetic evidences, the major natural auxin in plants, IAA (indole-3-acetic acid), is synthesized via two major pathways: Trp (Tryptophan)-independent (TI) and Trp-dependent (TD) pathways [5,22,68]. So far, the molecular components of the TI pathway have been poorly understood [69].

At present, it appears that the best understood IPA (indole pyruvic acid) pathway is the main TD pathway of auxin biosynthesis in *Arabidopsis thaliana* [48,70,71], in which TAA (tryptophan aminotransferase of Arabidopsis) family proteins catalyze the conversion of Trp to IPA [16,72–74], and YUC (YUCCA) flavin monooxygenase-like proteins catalyze the conversion of IPA to IAA [70,71,75]. Overexpression of YUCs, but not TAA family genes, leads to auxin overproduction, implying that the YUCs, rather than TAA family proteins, catalyze the rate-limiting step of the IPA pathway [70,76–78].

Besides TAA1/WEI8/SAV3/TIR2/CKRC1 (weak ethylene insensitive 8/shade avoidance 3/transport inhibitor response 2/cytokinin induced root curling 1) [16,72–74], the TAA family also includes two other homologous proteins: TAR1 (tryptophan aminotransferase related 1) and TAR2, which have overlapping functions [72]. YUCs belongs to a large gene family with 11 members in the *Arabidopsis* genome, which are functionally redundant to each other [75–81]. The importance of the IPA pathway in plant growth and development has made it the focus of research in recent years, and it is regulated by various developmental and environmental signals, including phytohormones [22,48,82].

Early definite evidence of the effect of cytokinin on auxin biosynthesis was reported by Jones et al. (2010), showing that cytokinin can induce auxin biosynthesis and that some signaling components of auxin and cytokinin are involved in this process; however, the molecular mechanism remains unclear [15]. Later, the authors found that cytokinin can induce the expression of both *TAA1* and *YUC8* genes to enhance auxin production [16,17]. In the adventitious root apex of *Arabidopsis*, cytokinin-mediated up-regulation of *YUC6* was found to be involved in the formation of the QC (Quiescent Center) [83]. Cytokinin-induced expression of *YUC1* and *YUC4* in the gynoecia primordium has been reported to ensure correct domain patterning [39].

Transcriptional activation of *TAA* and *YUC* family genes by cytokinin is dependent on cytokinin signaling transduction, as has been shown for *TAA1/CKRC1* [16] and *YUC8/CKRC2* [17] in *Arabidopsis* (Figure 1). Yan et al. (2017) found that type-B ARRs can directly bind two cis elements in the promoter and the second intron of *TAA1* to activate its transcription [18]. The AHKs-ARR1/12-mediated cytokinin signaling pathway is also reported to be necessary for cytokinin-induced up-regulation of *TAA1* and *YUC8*, and the PIF4 (phytochrome-interacting factors 4) is required for this upregulation. Transcription of *PIF4* itself is induced by cytokinin via the AHKs-ARR1/12 signaling pathway, indicating that PIF4 plays an essential role in mediating the regulatory effect of cytokinin on the transcriptions of *TAA1* and *YUC8* genes in the IPA pathway of auxin biosynthesis [17].



**Figure 1.** Auxin biosynthesis/metabolism and transport regulated by cytokinin.

Upstream of the IPA pathway, the two subunits of rate-limiting anthranilate synthase, *ASA1* (anthranilate synthase alpha-subunit 1)/*WEI2/CKRC6* and *ASB1* (anthranilate synthase beta-subunit 1)/*WEI7* in the Trp biosynthesis pathway [72,84], are specifically expressed in the root tip [72,73,79,85]. It has been shown that the transcriptional levels of *ASA1/WEI2/CKRC6* and *ASB1/WEI7* can be induced by cytokinin [15,85,86], and *ARR1* promotes auxin biosynthesis in the stem cell niche via the up-regulation of *ASB1/WEI7* in the root meristem (Figure 1) [85].

In another proposed but still somewhat obscure TD pathway named the IAOx (indole-3-acetaldoxime) pathway, the key enzymes of cytochrome P450 proteins *CYP79B2* and *CYP79B3* convert Trp to IAOx. Overexpression lines of these two genes showed auxin overproduction phenotypes and a high level of auxin; on the contrary, plants showed auxin-deficient phenotype and decreased endogenous auxin content when the enzyme function was lost [87,88]. Expressed in the root meristem [87], *CYP79B2* and *CYP79B3* are involved in root elongation [87,88]. Microarray and qRT-PCR results show that their transcription is activated when the plant is treated with cytokinin [15,89], and the result of ChIP-seq (chromatin immunoprecipitation sequencing) shows that *ARR1* can bind to a partial sequence of a *CYP79B3* gene after 3 h cytokinin treatment [90]. It is likely that the IAOx pathway could be another cytokinin-regulated auxin biosynthesis pathway.

Most recently, it was found that exogenous cytokinin can stimulate the expression of *CKRW2/HUB1* (cytokinin induced root waving 2/histone monoubiquitinate 1), which encodes an E3 ligase required for histone H2B mono-ubiquitination (H2Bub1) to promote the transcription of auxin biosynthetic genes *TRP2/TSB1* (tryptophan biosynthesis 2/tryptophan synthase beta-subunit 1), *ASB1/WEI7*, *YUC7* and *AMI1* (*amidase 1*) [91]. This discovery reveals an epigenetic mechanism of cytokinin-regulated IAA biosynthesis at the chromatin level.

In conclusion, cytokinin can control the level of auxin in roots by regulating local auxin biosynthesis, which is generally believed to be the basis of the gradient distribution of auxin [48,72,74,92], and is necessary for root growth and development [82,93,94].

#### 4. Cytokinin-Regulated IAA Conjugation and Degradation

In *Arabidopsis*, the GH3 (Gretchen Hagen 3) family belongs to a large gene family with three groups, of which group II (GH3.1-6, GH3.9 and GH3.17) has been shown to convert IAA to IAA amino acid conjugates [95–97]. The conjugation of amino acids with IAA is usually classified into two categories: one that can be converted to free IAA through hydrolysis and is considered to be related to the storage of auxin, such as IAA-Ala and IAA-Leu [98,99]; the other is believed to be related to the degradation of auxin, which can be irretrievably oxidized after formation and then degraded, such as IAA-Asp and IAA-Glu [100–102]. The process of IAA amino acid conjugation is generally considered to be associated with auxin homeostasis, which may play a role in cases where plant cells have to rapidly alter the relative amount of IAA in response to developmental and environmental changes [19,22,95,102–106].

The LRC (lateral root cap) is the outermost tissue of the root meristem [107]. If the LRC is lost, the size of the meristem will be greatly reduced [108,109]. This is because bPAT (basipetal PAT, from the root tip to the elongation zone) starts at the lateral root cap. If the LRC is lost, auxin transported from the root tip to the elongation zone will be disturbed. The defect of bPAT makes it impossible to establish a normal gradient distribution of auxin, so that the root meristem becomes smaller [21,110]. However, it is interesting to note that several members of group II GH3 (GH3.5, GH3.6 and GH3.17) are specifically expressed in the LRC [19–21], in which GH3.17 catalyzes IAA to IAA-Glu to participate in IAA degradation [19,21]. It has been reported that these three conjugation enzymes play a key role in controlling auxin flow in bPAT, as they determine the amount of auxin transport from the root tip to the elongation zone [19–21].

Surprisingly, *GH3.5*, *GH3.6* and *GH3.17* are downstream of type-B ARR1 in cytokinin signal transduction, and are targets of cytokinin–auxin antagonism [20,21]. Cytokinin suppresses bPAT by activating transcription of *GH3.5*, *GH3.6* and *GH3.17*, which convert free IAA to IAA amino acid conjugates, thus regulating the size of the root meristem (Figure 1) [20,21].

#### 5. Cytokinin-Regulated Intercellular Auxin Transport

The carriers that mediate auxin transport between cells contain three protein families: (1) AUX1/LAX (AUX1/LIKE AUX1) family proteins, responsible for the transport of auxin from the apoplast into the cell [111–115]; (2) PIN (PIN-formed) family proteins that mediate auxin output cells [116–120]; (3) ABCB/PGP/MDR (ATP-binding cassette protein subfamily B/P-Glyco protein/multidrug resistance) family proteins, involved in the ATP-driven influx or efflux of auxin [121,122].

Of these three families, only AUX1/LAX influx and PIN efflux carriers are involved in PAT machinery, directing the flow of auxin from the shoot acropetally through the stele toward the root tip (aPAT, acropetal PAT). From here it is basipetally redistributed via the epidermis to the elongation zone (bPAT) [115,116,120,123–127]. The pattern of expression of the various AUX1/LAX and PIN genes and the localization of them on specific cell faces play a key role in PAT machinery to determine the distribution of auxin in plant tissues [115,116,120,123–127]. Unlike AUX1/LAX influx and PIN efflux carriers,

the ABCB/PGP/MDR family proteins have also been shown to act as auxin transporters to mediate auxin in and out of cells; however, because they are uniformly localized in the cell, they are considered to be unrelated to PAT [128,129].

In the last 10 years, studies on cytokinin-regulated plant development have revealed that a number of processes are involved in cytokinin interaction with PAT (e.g., root and shoot apical meristem activity maintenance, lateral root organogenesis, vasculature differentiation, or phyllotaxis [11,26,47,130,131]). In primary roots, previous studies suggested that cytokinin inhibition of cell expansion depended on cytokinin-induced ethylene biosynthesis [132]. The inhibition of root cell elongation requires ethylene regulated transport-dependent auxin distribution [27,133]. Although the role of ethylene in the cytokinin response has been demonstrated, the direct regulation of PAT by cytokinin is more important for root growth and development.

### 5.1. PINs Efflux Carriers

In *Arabidopsis thaliana*, according to the length of the hydrophilic loop in the middle of the polypeptide chain, the PINs family is divided into two subfamilies: as auxin efflux carriers, PIN1, PIN2, PIN3, PIN4 and PIN7 contain a long hydrophilic loop and are located in the PM (plasma membrane) [117,119,120], while PIN5, PIN6 and PIN8 with a short hydrophilic loop are mainly located in the ER (endoplasmic reticulum), which are involved in intracellular auxin transport [117,119,120,134]. All of the PIN efflux carriers are expressed and active in the root tip and perform their respective functions [116,117,119,120,129,135].

Cytokinin has been shown to influence cell-to-cell auxin transport by regulating the expression of several PIN genes, thereby modulating auxin distribution, which is essential for root development [11,26,131,136,137]. In *Arabidopsis* roots, through the cytokinin receptor AHK3 and the downstream signaling components ARR1 and ARR12, cytokinin has been shown to activate SHY2 (short hypocotyl 2), which is a member of the AUX/IAA (Auxin/Indole-3-Acetic Acid) protein family that heterodimerizes with ARFs (auxin response factors), preventing the activation of auxin responses. Therefore, as a downstream gene of AFRs, the expression of *PIN1*, *PIN3* and *PIN7* was inhibited when SHY2 was activated (Figure 1) [11,138].

Some CRFs also directly fine-tune PIN expression, providing a direct regulatory link between cytokinin signaling and the auxin transport machinery. Plants lacking CRF activity show developmental pattern aberrations consistent with abnormal auxin distribution. Removal of specific cis-regulatory elements (PCRE (PIN cytokinin response element) domain 5'-AGCAGAC-3'-like motif) effectively uncouples PIN1 and PIN7 transcription from the CRF-dependent regulation, and attenuates plant cytokinin sensitivity (Figure 1) [137]. Furthermore, the bHLH TF SPATULA enables cytokinin signaling, and activates the expression of *PIN3* [139].

Besides transcriptional regulation, cytokinin also negatively regulates PINs at the post-transcriptional level [140]. Cytokinin can affect endomembrane trafficking of PIN1, PIN3 and PIN7 to redirect them for lytic degradation in vacuoles to reduce their abundance on the plasma membrane [141], and this function relies on canonical cytokinin signaling components, including the cytokinin receptor AHK4/CRE1 and some type-B ARRs [47]. PIN1 phosphorylation status is also involved (Figure 1) [142].

The complexity of cytokinin effects on PINs to regulate PAT in various cells/tissues/organs and developmental stages by various mechanisms has led to some confusing or even seemingly contradictory results. For example, in studying the effect of exogenous cytokinin on the transcription of PIN1, cytokinin was found to inhibit PIN1 transcription by using a 2-mm root tip with meristem/transition/elongation zones as the material [140], but was reported to have no such effect on a 0.5-mm root tip mainly with meristem zones [11,26,140]. In fact, cytokinin still causes PIN1 inactivation in the meristem, which depends on cytokinin-induced post-transcriptional regulation [140]. As another example, cytokinin down-regulates PIN1 and PIN3 proteins in primary roots to inhibit



aPAT [11,26,136], but promotes the accumulation of PIN3, PIN4, and PIN7 in shoots, thereby coordinating bud outgrowth and branching [47].

Despite these complications, at present, it is generally believed that cytokinin down-regulates PAT by inhibiting all PIN efflux carriers except PIN7 in primary root tips [11,26,93,136,137,140,141].

### 5.2. AUX1/LAX Influx Carriers

In *Arabidopsis thaliana*, AUX1/LAX influx carriers are encoded by a small multigene family comprised of four members: AUX1, LAX1, LAX2, and LAX3 [111–115]. They display reasonably distinct expression patterns and are suggested to participate in different developmental processes [111]. Of all the members, except for LAX1, which is not involved in root development [111], the AUX1 gene is mainly expressed in the LRC, epidermal and phloem tissues near the root tip [113,143], and has been shown to play a role in gravitropism [143]; both AUX1 and LAX3 are shown to regulate lateral root development [112], and LAX2 is strongly expressed in the QC and the LRC [111], where it plays a key role in maintaining the stem cell fate surrounding the QC [144]. It was found that disruption of the LAX2 gene results in a phenotype similar to that observed in type-A ARR mutants, such as increased division of cells in the QC [144]. This is because auxin influx carriers, LAX2 genes, act downstream of cytokinin in the root tip, whose transcription is suppressed by cytokinin [27,144]. The decrease in AUX1 and LAX2 expression in response to cytokinin requires cytokinin response transcriptional effector type-B ARRs, which mediate the primary transcriptional response to cytokinin (Figure 1) [27,144,145]. CHIP assays showed that the AUX1 gene was enriched for extended type-B ARR12 binding motifs in intron 8 [27,145], and type-B ARR1 was found to bind directly to intron 2, intron 4 and 1.2 kb upstream motifs of the LAX2 gene [144]. These studies indicate that cytokinin response transcriptional effector type-B ARRs directly down-regulate the expression of AUX1/LAX influx carriers.

## 6. Cytokinin-Regulated Intracellular Auxin Transport

In addition to the above-mentioned PINs for intercellular PAT, the auxin carrier proteins for intracellular auxin transport include ER-localized PIN5, PIN6, PIN8, and other PILSs (PIN-like proteins), which are likely older than PINs by phylogenetic analysis [120,146–148]. There are seven known members of the PILS family. Although the PILS proteins share only 10–18% of their sequence with PIN proteins, they are topologically similar [147–149]. Members of the PILS family are identified by the presence of an auxin carrier domain that spans almost the entire length of the PILS proteins; therefore, PILS proteins still have the ability to transport auxin across the membrane [120,146].

Compared with the auxin efflux PINs located on the plasma membrane, which are involved in the intercellular transport of auxin, ER-localized PINs and PILSs mediate the intracellular transport of auxin [120,134,150–154]. ER-localized PINs are speculated to mediate auxin flow into (PIN5) or out (PIN8) of the ER lumen [120,152,154], or hypothetically from the ER lumen into the nucleus (PIN6 and PIN8) to open the auxin downstream genes' transcription [150,152]. Like PIN5, the expression of PILS2 and PILS5 transporters causes cytosolic auxin to be transported into the ER lumen, leading to reduced transcriptional regulation of downstream genes by auxin in the nucleus, thus reducing auxin signals and cell sensitivity to auxin [120,146–148,155,156].

PIN5 is expressed in the vasculature of the mature root zone [157] and epidermis of the meristem zone [21]; PILS2 and PILS5 showed a particular overlapping expression in the root transition zone [146]. In root growth and development, PIN5, PILS2 and PILS5 play a negative role in primary root elongation [21,146,148]. The roots of PIN5, PILS2, or PILS5 gain-of-function mutants become shorter; on the contrary, the roots of loss-of-function mutants become longer [21,146,148]. This is because ER-localization auxin transport carriers, PIN5 and PILSs negatively regulate PAT and auxin signaling [21,134,146,147,154–156]. Interestingly, unlike PINs involved in intercellular transport, transcription of PIN5 and PILS5

is induced by cytokinin (Figure 1) [15,21,158,159], suggesting that PIN5 and PILS5 are other targets of cytokinin–auxin antagonism besides auxin conjugation enzymes GH3.5, GH3.6 and GH3.17. In other words, the process of cytokinin activating *PIN5* and *PILS5* expression reduces the amount of auxin transport to root elongation zones through intracellular auxin accumulation in bPAT transport cells. A recent paper reported that ARR1 binds directly to the *PIN5* promoter to mediate cytokinin induction of *PIN5* expression (Figure 1) [21]. Furthermore, it has been reported that PIN5-mediated intracellular auxin accumulation and GH3.17-mediated auxin conjugation with Glu are inextricably linked, which together regulate auxin homeostasis and signal transduction [21,160]; however, the details require further study.

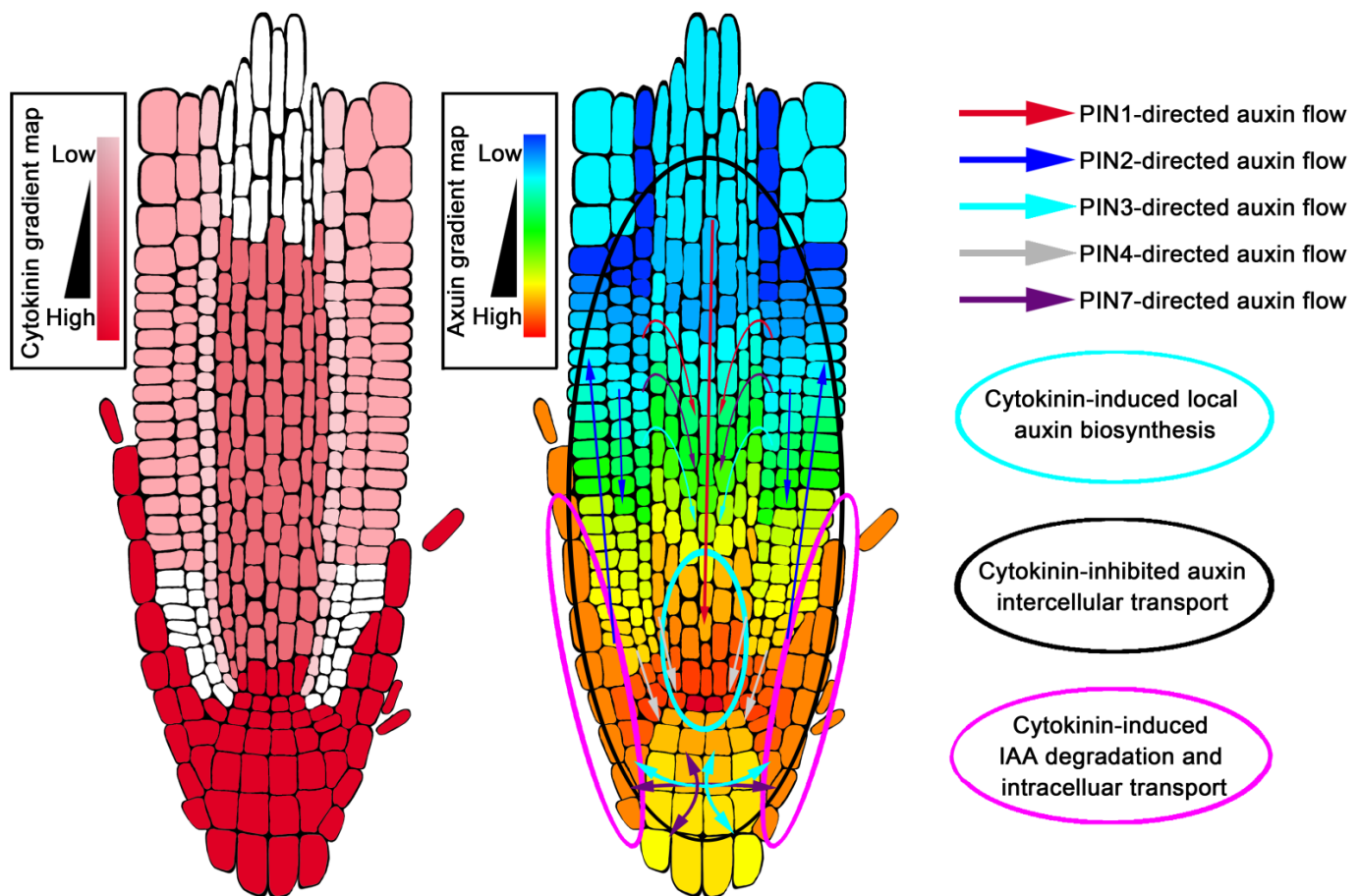
## 7. Concluding Remarks

Once the seed germinates, the root meristem, which is derived from the proximal stem cells, proliferates and expands rapidly. After approximately 5 days of growth and development, the meristem of the root tip reaches a stable size, by which root growth is sustained. Cytokinin and auxin interactions play key roles in controlling the balance between the rate of cell division and differentiation, which is crucial for the maintenance of root meristems [11,19,26,161]. Here, we reviewed the cytokinin-mediated regulation on the components involved in auxin biosynthesis/metabolism, polar auxin transport, and intracellular transport of auxin in root tips (Figure 1). These processes are all related to establish the gradient distribution of auxin in root tips. They work together to control the size of the meristem, which is significantly reduced in *taa1/ckrc1* [16], *yuc8/ckrc2* [17], and the triple mutant *pin1 pin3 pin7* [11], but increased in *pin5-3* [21], *gh3.5*, *gh3.6* and *gh3.17* [19–21].

According to the current auxin gradient distribution model, auxin concentration is maximal at the QC of the root tip, but minimal at the boundary of the transition zone (Figure 2) [19,162]. The auxin maximum is very important for the maintenance of the QC and stem cell fate surrounding the QC [162], while the auxin minimum at the boundary of the transition zone acts as a signal to control the developmental switch from cell division to cell differentiation [19]. Earlier studies believed that the establishment of the maximum value of auxin in the root tip depended on the auxin transport mediated by PINs. This auxin maximum involves three transport processes, which are aPAT, bPAT and reflux of auxin. The auxin reflux redistributes auxin to form a characteristic gradient, and auxin is maximally located at the QC [162]. IAA3/SHY2, a negative regulator of auxin signal transduction, is a direct transcriptional target downstream of cytokinin signal AHK3-ARR1,12, which is one of the sites where the two hormones interact [11,138]. Cytokinin signaling suppresses auxin efflux carriers PIN1, PIN3 and PIN7 expressions through IAA3/SHY2 upregulation [11,26], and at the same time, promotes PIN1, PIN3 and PIN4 protein degradation to decrease PIN abundance at the post-transcriptional level [140]. Thus, cytokinin controls the flow of auxin to the root tip through the precise regulation of PINs. This process not only determines auxin levels that generate a minimum in the vascular tissue of the transition zone, but also determines the maximum value of auxin in the QC of the root tip (Figure 2) [11,19,26,140,163].

In addition, the auxin reflux mechanism gradually decreases auxin when it is transported along the external tissues of roots from the top to the base [162]; however, the formation of the external tissue auxin gradient is also regulated by cytokinin-mediated auxin degradation [19]. Several years ago, fluorescence-activated cell sorting of green fluorescent protein (GFP)-marked cell types, combined with solid-phase microextraction and an ultra-high-sensitivity mass spectrometry (MS) assay, was applied to analyze the levels of cytokinin in each tissue of the root tip (Figure 2) [164]. Cytokinin was found to be mainly concentrated in the lateral root cap, columella, columella initials, QC cells, and in the epidermis of the root tip, forming a gradient down to a maxima at the root cap [164]. Several components involved in cytokinin regulating auxin gradient distribution are specifically expressed in high cytokinin level regions of the root. When the auxin in

the root tip was transported from the LRC to the elongation zone through bPAT, cytokinin could up-regulate the expression of genes *GH3.5*, *GH3.6*, *GH3.17* and *PIN5* in the LRC and the epidermis near the root tip to gradually reduce the amount of auxin transported by bPAT (Figure 2) [19,21,160].



**Figure 2.** Cytokinin-controlled gradient distribution of auxin in *Arabidopsis* root tip.

Although calculated models based on the PINs and other auxin transporters indicate that the transport and redistribution of auxin from the above-ground parts is sufficient to produce the auxin gradient and auxin maximum in the root [165], the effect of local auxin biosynthesis on growth and development cannot be ignored [81,82,94,163]. Disturbing local auxin biosynthesis in the root tip will cause developmental defects, such as the small meristem and gravitropic defect [16,17,86]. There are evidences that auxin overproduction in shoots cannot completely rescue root auxin deficiency phenotypes, highlighting the importance of local auxin biosynthesis for root development [79]. In Section 3, we reviewed several processes by which local auxin biosynthesis is activated by cytokinin. At present, little is known about the physiological functions of these processes. We speculate that the local auxin biosynthesis induced by cytokinin may be a compensation mechanism of auxin. The establishment and maintenance of the gradient distribution of auxin in the root tip depends on auxin transport and reflux, and auxin is constantly attenuated by the downstream signal of cytokinin in the flow (Sections 4 and 6). This compensation mechanism can offset the attenuated auxin, thereby maintaining the stability of the meristem.

In summary, recent studies highlight the important role of the cytokinin-controlled gradient distribution of auxin in root growth and development. However, some questions remain. For example, there are many mechanisms by which cytokinins regulate the gradient distribution of auxin; how do plants balance the local auxin biosynthesis, degradation,



intercellular transport and intracellular transport in this process? Are these processes connected or dissected during plant growth and development?

**Author Contributions:** L.W. and G.-Q.G. drafted, wrote, and edited this review. J.-L.W. and X.-F.L. participated in the discussion of this review. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was supported by the National Natural Science Foundation of China (31970713), Fundamental Research Funds for the Central Universities (lzujbky-2020-33) and Natural Science Foundation of Gansu Province (20JR5RA270).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

- Enders, T.A.; Strader, L.C. Auxin activity: Past, present, and future. *Am. J. Bot.* **2015**, *102*, 180–196. [[CrossRef](#)] [[PubMed](#)]
- Lavy, M.; Estelle, M. Mechanisms of auxin signaling. *Development* **2016**, *143*, 3226–3229. [[CrossRef](#)] [[PubMed](#)]
- Sabatini, S.; Beis, D.; Wolkenfelt, H.; Murfett, J.; Guilfoyle, T.; Malamy, J.; Benfey, P.; Leysner, O.; Bechtold, N.; Weisbeek, P.; et al. An auxin-dependent distal organizer of pattern and polarity in the Arabidopsis root. *Cell* **1999**, *99*, 463–472. [[CrossRef](#)]
- Weijers, D.; Nemhauser, J.; Yang, Z. Auxin: Small molecule, big impact. *J. Exp. Bot.* **2018**, *69*, 133–136. [[CrossRef](#)] [[PubMed](#)]
- Woodward, A.W.; Bartel, B. Auxin: Regulation, action, and interaction. *Ann. Bot.* **2005**, *95*, 707–735. [[CrossRef](#)]
- Pacifici, E.; Polverari, L.; Sabatini, S. Plant hormone cross-talk: The pivot of root growth. *J. Exp. Bot.* **2015**, *66*, 1113–1121. [[CrossRef](#)]
- Petricka, J.J.; Winter, C.M.; Benfey, P.N. Control of Arabidopsis root development. *Annu Rev. Plant Biol.* **2012**, *63*, 563–590. [[CrossRef](#)]
- Miller, C.O.; Skoog, F.; Von Saltza, M.H.; Strong, F. Kinetin, a cell division factor from deoxyribonucleic acid. *J. Am. Chem. Soc.* **1955**, *77*, 1392. [[CrossRef](#)]
- Miller, C.O.; Skoog, F.; Okomura, F.S.; von Saltza, M.H.; Strong, F.M. Isolation, structure and synthesis of kinetin, a substance promoting cell division. *J. Am. Chem. Soc.* **1956**, *78*, 1345–1350. [[CrossRef](#)]
- Skoog, F.; Miller, C.O. Chemical regulation of growth and organ formation in plant tissues cultured in vitro. *Symp. Soc. Exp. Biol.* **1957**, *11*, 118–130.
- Dello Ioio, R.; Nakamura, K.; Moubayidin, L.; Perilli, S.; Taniguchi, M.; Morita, M.T.; Aoyama, T.; Costantino, P.; Sabatini, S. A genetic framework for the control of cell division and differentiation in the root meristem. *Science* **2008**, *322*, 1380–1384. [[CrossRef](#)]
- Kieber, J.J.; Schaller, G.E. Cytokinin signaling in plant development. *Development* **2018**, *145*, dev149344. [[CrossRef](#)]
- Tanaka, H.; Dhonukshe, P.; Brewer, P.B.; Friml, J. Spatiotemporal asymmetric auxin distribution: A means to coordinate plant development. *Cell Mol. Life Sci.* **2006**, *63*, 2738–2754. [[CrossRef](#)] [[PubMed](#)]
- Vieten, A.; Sauer, M.; Brewer, P.B.; Friml, J. Molecular and cellular aspects of auxin-transport-mediated development. *Trends Plant Sci.* **2007**, *12*, 160–168. [[CrossRef](#)] [[PubMed](#)]
- Jones, B.; Gunneras, S.A.; Petersson, S.V.; Tarkowski, P.; Graham, N.; May, S.; Dolezal, K.; Sandberg, G.; Ljung, K. Cytokinin regulation of auxin synthesis in Arabidopsis involves a homeostatic feedback loop regulated via auxin and cytokinin signal transduction. *Plant Cell* **2010**, *22*, 2956–2969. [[CrossRef](#)]
- Zhou, Z.Y.; Zhang, C.G.; Wu, L.; Zhang, C.G.; Chai, J.; Wang, M.; Jha, A.; Jia, P.F.; Cui, S.J.; Yang, M.; et al. Functional characterization of the CKRC1/TAA1 gene and dissection of hormonal actions in the Arabidopsis root. *Plant J.* **2011**, *66*, 516–527. [[CrossRef](#)]
- Di, D.W.; Wu, L.; Zhang, L.; An, C.W.; Zhang, T.Z.; Luo, P.; Gao, H.H.; Kriechbaumer, V.; Guo, G.Q. Functional roles of Arabidopsis CKRC2/YUCCA8 gene and the involvement of PIF4 in the regulation of auxin biosynthesis by cytokinin. *Sci. Rep.* **2016**, *6*, 36866. [[CrossRef](#)] [[PubMed](#)]
- Yan, Z.; Liu, X.; Ljung, K.; Li, S.; Zhao, W.; Yang, F.; Wang, M.; Tao, Y. Type B Response Regulators Act As Central Integrators in Transcriptional Control of the Auxin Biosynthesis Enzyme TAA1. *Plant Physiol.* **2017**, *175*, 1438–1454. [[CrossRef](#)] [[PubMed](#)]
- Di Mambro, R.; De Ruvo, M.; Pacifici, E.; Salvi, E.; Sozzani, R.; Benfey, P.N.; Busch, W.; Novak, O.; Ljung, K.; Di Paola, L.; et al. Auxin minimum triggers the developmental switch from cell division to cell differentiation in the Arabidopsis root. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, E7641–E7649. [[CrossRef](#)]
- Pierdonati, E.; Unterholzner, S.J.; Salvi, E.; Svolacchia, N.; Bertolotti, G.; Dello Ioio, R.; Sabatini, S.; Di Mambro, R. Cytokinin-Dependent Control of GH3 Group II Family Genes in the Arabidopsis Root. *Plants* **2019**, *8*, 94. [[CrossRef](#)]
- Di Mambro, R.; Svolacchia, N.; Dello Ioio, R.; Pierdonati, E.; Salvi, E.; Pedrazzini, E.; Vitale, A.; Perilli, S.; Sozzani, R.; Benfey, P.N.; et al. The Lateral Root Cap Acts as an Auxin Sink that Controls Meristem Size. *Curr. Biol.* **2019**, *29*, 1199–1205.e4. [[CrossRef](#)]

22. Casanova-Saez, R.; Mateo-Bonmati, E.; Ljung, K. Auxin Metabolism in Plants. *Cold Spring Harb. Perspect. Biol.* **2021**, *13*, a039867. [[CrossRef](#)] [[PubMed](#)]
23. Liu, J.; Moore, S.; Chen, C.; Lindsey, K. Crosstalk Complexities between Auxin, Cytokinin, and Ethylene in Arabidopsis Root Development: From Experiments to Systems Modeling, and Back Again. *Mol. Plant* **2017**, *10*, 1480–1496. [[CrossRef](#)]
24. Moore, S.; Zhang, X.; Mudge, A.; Rowe, J.H.; Topping, J.F.; Liu, J.; Lindsey, K. Spatiotemporal modelling of hormonal crosstalk explains the level and patterning of hormones and gene expression in Arabidopsis thaliana wild-type and mutant roots. *New Phytol.* **2015**, *207*, 1110–1122. [[CrossRef](#)] [[PubMed](#)]
25. Muraro, D.; Larrieu, A.; Lucas, M.; Chopard, J.; Byrne, H.; Godin, C.; King, J. A multi-scale model of the interplay between cell signalling and hormone transport in specifying the root meristem of Arabidopsis thaliana. *J. Theor. Biol.* **2016**, *404*, 182–205. [[CrossRef](#)]
26. Ruzicka, K.; Simaskova, M.; Duclercq, J.; Petrasek, J.; Zazimalova, E.; Simon, S.; Friml, J.; Van Montagu, M.C.; Benkova, E. Cytokinin regulates root meristem activity via modulation of the polar auxin transport. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 4284–4289. [[CrossRef](#)] [[PubMed](#)]
27. Street, I.H.; Mathews, D.E.; Yamburkenko, M.V.; Sorooshzadeh, A.; John, R.T.; Swarup, R.; Bennett, M.J.; Kieber, J.J.; Schaller, G.E. Cytokinin acts through the auxin influx carrier AUX1 to regulate cell elongation in the root. *Development* **2016**, *143*, 3982–3993. [[CrossRef](#)]
28. Argueso, C.T.; Ferreira, F.J.; Kieber, J.J. Environmental perception avenues: The interaction of cytokinin and environmental response pathways. *Plant Cell Environ.* **2009**, *32*, 1147–1160. [[CrossRef](#)]
29. Bishopp, A.; Benkova, E.; Helariutta, Y. Sending mixed messages: Auxin-cytokinin crosstalk in roots. *Curr. Opin. Plant Biol.* **2011**, *14*, 10–16. [[CrossRef](#)]
30. Chandler, J.W.; Werr, W. Cytokinin-auxin crosstalk in cell type specification. *Trends Plant Sci.* **2015**, *20*, 291–300. [[CrossRef](#)]
31. Del Bianco, M.; Giustini, L.; Sabatini, S. Spatiotemporal changes in the role of cytokinin during root development. *New Phytol.* **2013**, *199*, 324–338. [[CrossRef](#)]
32. Domagalska, M.A.; Leyser, O. Signal integration in the control of shoot branching. *Nat. Rev. Mol. Cell Biol.* **2011**, *12*, 211–221. [[CrossRef](#)] [[PubMed](#)]
33. El-Showk, S.; Ruonala, R.; Helariutta, Y. Crossing paths: Cytokinin signalling and crosstalk. *Development* **2013**, *140*, 1373–1383. [[CrossRef](#)]
34. Hwang, I.; Sheen, J.; Muller, B. Cytokinin signaling networks. *Annu. Rev. Plant Biol.* **2012**, *63*, 353–380. [[CrossRef](#)] [[PubMed](#)]
35. Jing, H.; Strader, L.C. Interplay of Auxin and Cytokinin in Lateral Root Development. *Int. J. Mol. Sci.* **2019**, *20*, 486. [[CrossRef](#)] [[PubMed](#)]
36. Kieber, J.J.; Schaller, G.E. Cytokinins. *Arab. Book* **2014**, *12*, e0168. [[CrossRef](#)] [[PubMed](#)]
37. Kurepa, J.; Shull, T.E.; Smalle, J.A. Antagonistic activity of auxin and cytokinin in shoot and root organs. *Plant Direct* **2019**, *3*, e00121. [[CrossRef](#)] [[PubMed](#)]
38. Moubayidin, L.; Di Mambro, R.; Sabatini, S. Cytokinin-auxin crosstalk. *Trends Plant Sci.* **2009**, *14*, 557–562. [[CrossRef](#)]
39. Muller, C.J.; Larsson, E.; Spichal, L.; Sundberg, E. Cytokinin-Auxin Crosstalk in the Gynoecial Primordium Ensures Correct Domain Patterning. *Plant Physiol.* **2017**, *175*, 1144–1157. [[CrossRef](#)]
40. Naseem, M.; Dandekar, T. The role of auxin-cytokinin antagonism in plant-pathogen interactions. *PLoS Pathog.* **2012**, *8*, e1003026. [[CrossRef](#)] [[PubMed](#)]
41. O'Brien, J.A.; Benkova, E. Cytokinin cross-talking during biotic and abiotic stress responses. *Front. Plant Sci.* **2013**, *4*, 451. [[CrossRef](#)]
42. Pernisova, M.; Kuderova, A.; Hejatkó, J. Cytokinin and auxin interactions in plant development: Metabolism, signalling, transport and gene expression. *Curr. Protein Pept. Sci.* **2011**, *12*, 137–147. [[CrossRef](#)]
43. Schaller, G.E.; Bishopp, A.; Kieber, J.J. The yin-yang of hormones: Cytokinin and auxin interactions in plant development. *Plant Cell* **2015**, *27*, 44–63. [[CrossRef](#)]
44. Su, Y.H.; Liu, Y.B.; Zhang, X.S. Auxin-cytokinin interaction regulates meristem development. *Mol. Plant* **2011**, *4*, 616–625. [[CrossRef](#)] [[PubMed](#)]
45. Tognetti, V.B.; Bielach, A.; Hrtyan, M. Redox regulation at the site of primary growth: Auxin, cytokinin and ROS crosstalk. *Plant Cell Environ.* **2017**, *40*, 2586–2605. [[CrossRef](#)] [[PubMed](#)]
46. Vanstraelen, M.; Benkova, E. Hormonal interactions in the regulation of plant development. *Annu. Rev. Cell Dev. Biol.* **2012**, *28*, 463–487. [[CrossRef](#)] [[PubMed](#)]
47. Waldie, T.; Leyser, O. Cytokinin Targets Auxin Transport to Promote Shoot Branching. *Plant Physiol.* **2018**, *177*, 803–818. [[CrossRef](#)]
48. Zhao, Y. Auxin biosynthesis. *Arab. Book* **2014**, *12*, e0173. [[CrossRef](#)] [[PubMed](#)]
49. To, J.P.; Kieber, J.J. Cytokinin signaling: Two-components and more. *Trends Plant Sci.* **2008**, *13*, 85–92. [[CrossRef](#)]
50. Inoue, T.; Higuchi, M.; Hashimoto, Y.; Seki, M.; Kobayashi, M.; Kato, T.; Tabata, S.; Shinozaki, K.; Kakimoto, T. Identification of CRE1 as a cytokinin receptor from Arabidopsis. *Nature* **2001**, *409*, 1060–1063. [[CrossRef](#)] [[PubMed](#)]
51. Suzuki, T.; Miwa, K.; Ishikawa, K.; Yamada, H.; Aiba, H.; Mizuno, T. The Arabidopsis sensor His-kinase, AHK4, can respond to cytokinins. *Plant Cell Physiol.* **2001**, *42*, 107–113. [[CrossRef](#)] [[PubMed](#)]
52. Ueguchi, C.; Sato, S.; Kato, T.; Tabata, S. The AHK4 gene involved in the cytokinin-signaling pathway as a direct receptor molecule in Arabidopsis thaliana. *Plant Cell Physiol.* **2001**, *42*, 751–755. [[CrossRef](#)]

53. Yamada, H.; Suzuki, T.; Terada, K.; Takei, K.; Ishikawa, K.; Miwa, K.; Yamashino, T.; Mizuno, T. The Arabidopsis AHK4 histidine kinase is a cytokinin-binding receptor that transduces cytokinin signals across the membrane. *Plant Cell Physiol.* **2001**, *42*, 1017–1023. [[CrossRef](#)] [[PubMed](#)]
54. Hutchison, C.E.; Li, J.; Argueso, C.; Gonzalez, M.; Lee, E.; Lewis, M.W.; Maxwell, B.B.; Perdue, T.D.; Schaller, G.E.; Alonso, J.M.; et al. The Arabidopsis histidine phosphotransfer proteins are redundant positive regulators of cytokinin signaling. *Plant Cell* **2006**, *18*, 3073–3087. [[CrossRef](#)] [[PubMed](#)]
55. Miyata, S.; Urao, T.; Yamaguchi-Shinozaki, K.; Shinozaki, K. Characterization of genes for two-component phosphorelay mediators with a single HPt domain in Arabidopsis thaliana. *FEBS Lett.* **1998**, *437*, 11–14. [[CrossRef](#)]
56. Suzuki, T.; Imamura, A.; Ueguchi, C.; Mizuno, T. Histidine-containing phosphotransfer (HPt) signal transducers implicated in His-to-Asp phosphorelay in Arabidopsis. *Plant Cell Physiol.* **1998**, *39*, 1258–1268. [[CrossRef](#)]
57. Tanaka, Y.; Suzuki, T.; Yamashino, T.; Mizuno, T. Comparative studies of the AHP histidine-containing phosphotransmitters implicated in His-to-Asp phosphorelay in Arabidopsis thaliana. *Biosci. Biotechnol. Biochem.* **2004**, *68*, 462–465. [[CrossRef](#)] [[PubMed](#)]
58. Argyros, R.D.; Mathews, D.E.; Chiang, Y.H.; Palmer, C.M.; Thibault, D.M.; Etheridge, N.; Argyros, D.A.; Mason, M.G.; Kieber, J.J.; Schaller, G.E. Type B response regulators of Arabidopsis play key roles in cytokinin signaling and plant development. *Plant Cell* **2008**, *20*, 2102–2116. [[CrossRef](#)]
59. Ishida, K.; Yamashino, T.; Yokoyama, A.; Mizuno, T. Three type-B response regulators, ARR1, ARR10 and ARR12, play essential but redundant roles in cytokinin signal transduction throughout the life cycle of Arabidopsis thaliana. *Plant Cell Physiol.* **2008**, *49*, 47–57. [[CrossRef](#)]
60. Mason, M.G.; Mathews, D.E.; Argyros, D.A.; Maxwell, B.B.; Kieber, J.J.; Alonso, J.M.; Ecker, J.R.; Schaller, G.E. Multiple type-B response regulators mediate cytokinin signal transduction in Arabidopsis. *Plant Cell* **2005**, *17*, 3007–3018. [[CrossRef](#)]
61. Yokoyama, A.; Yamashino, T.; Amano, Y.; Tajima, Y.; Imamura, A.; Sakakibara, H.; Mizuno, T. Type-B ARR transcription factors, ARR10 and ARR12, are implicated in cytokinin-mediated regulation of protoxylem differentiation in roots of Arabidopsis thaliana. *Plant Cell Physiol.* **2007**, *48*, 84–96. [[CrossRef](#)] [[PubMed](#)]
62. Kiba, T.; Yamada, H.; Sato, S.; Kato, T.; Tabata, S.; Yamashino, T.; Mizuno, T. The type-A response regulator, ARR15, acts as a negative regulator in the cytokinin-mediated signal transduction in Arabidopsis thaliana. *Plant Cell Physiol.* **2003**, *44*, 868–874. [[CrossRef](#)] [[PubMed](#)]
63. Lee, D.J.; Park, J.Y.; Ku, S.J.; Ha, Y.M.; Kim, S.; Kim, M.D.; Oh, M.H.; Kim, J. Genome-wide expression profiling of ARABIDOPSIS RESPONSE REGULATOR 7 (ARR7) overexpression in cytokinin response. *Mol. Genet. Genom.* **2007**, *277*, 115–137. [[CrossRef](#)] [[PubMed](#)]
64. To, J.P.; Deruere, J.; Maxwell, B.B.; Morris, V.F.; Hutchison, C.E.; Ferreira, F.J.; Schaller, G.E.; Kieber, J.J. Cytokinin regulates type-A Arabidopsis Response Regulator activity and protein stability via two-component phosphorelay. *Plant Cell* **2007**, *19*, 3901–3914. [[CrossRef](#)] [[PubMed](#)]
65. To, J.P.; Haberer, G.; Ferreira, F.J.; Deruere, J.; Mason, M.G.; Schaller, G.E.; Alonso, J.M.; Ecker, J.R.; Kieber, J.J. Type-A Arabidopsis response regulators are partially redundant negative regulators of cytokinin signaling. *Plant Cell* **2004**, *16*, 658–671. [[CrossRef](#)] [[PubMed](#)]
66. Rashotte, A.M.; Carson, S.D.; To, J.P.; Kieber, J.J. Expression profiling of cytokinin action in Arabidopsis. *Plant Physiol.* **2003**, *132*, 1998–2011. [[CrossRef](#)]
67. Rashotte, A.M.; Mason, M.G.; Hutchison, C.E.; Ferreira, F.J.; Schaller, G.E.; Kieber, J.J. A subset of Arabidopsis AP2 transcription factors mediates cytokinin responses in concert with a two-component pathway. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 11081–11085. [[CrossRef](#)]
68. Normanly, J. Approaching cellular and molecular resolution of auxin biosynthesis and metabolism. *Cold Spring Harb. Perspect. Biol.* **2010**, *2*, a001594. [[CrossRef](#)]
69. Nonhebel, H.M. Tryptophan-Independent Indole-3-Acetic Acid Synthesis: Critical Evaluation of the Evidence. *Plant Physiol.* **2015**, *169*, 1001–1005. [[CrossRef](#)]
70. Mashiguchi, K.; Tanaka, K.; Sakai, T.; Sugawara, S.; Kawaide, H.; Natsume, M.; Hanada, A.; Yaeno, T.; Shirasu, K.; Yao, H.; et al. The main auxin biosynthesis pathway in Arabidopsis. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 18512–18517. [[CrossRef](#)]
71. Won, C.; Shen, X.; Mashiguchi, K.; Zheng, Z.; Dai, X.; Cheng, Y.; Kasahara, H.; Kamiya, Y.; Chory, J.; Zhao, Y. Conversion of tryptophan to indole-3-acetic acid by TRYPTOPHAN AMINOTRANSFERASES OF ARABIDOPSIS and YUCCAs in Arabidopsis. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 18518–18523. [[CrossRef](#)] [[PubMed](#)]
72. Stepanova, A.N.; Robertson-Hoyt, J.; Yun, J.; Benavente, L.M.; Xie, D.Y.; Dolezal, K.; Schlereth, A.; Jurgens, G.; Alonso, J.M. TAA1-mediated auxin biosynthesis is essential for hormone crosstalk and plant development. *Cell* **2008**, *133*, 177–191. [[CrossRef](#)] [[PubMed](#)]
73. Tao, Y.; Ferrer, J.L.; Ljung, K.; Pojer, F.; Hong, F.; Long, J.A.; Li, L.; Moreno, J.E.; Bowman, M.E.; Ivans, L.J.; et al. Rapid synthesis of auxin via a new tryptophan-dependent pathway is required for shade avoidance in plants. *Cell* **2008**, *133*, 164–176. [[CrossRef](#)] [[PubMed](#)]
74. Yamada, M.; Greenham, K.; Prigge, M.J.; Jensen, P.J.; Estelle, M. The TRANSPORT INHIBITOR RESPONSE2 gene is required for auxin synthesis and diverse aspects of plant development. *Plant Physiol.* **2009**, *151*, 168–179. [[CrossRef](#)] [[PubMed](#)]

75. Stepanova, A.N.; Yun, J.; Robles, L.M.; Novak, O.; He, W.; Guo, H.; Ljung, K.; Alonso, J.M. The Arabidopsis YUCCA1 flavin monooxygenase functions in the indole-3-pyruvic acid branch of auxin biosynthesis. *Plant Cell* **2011**, *23*, 3961–3973. [[CrossRef](#)]
76. Cheng, Y.; Dai, X.; Zhao, Y. Auxin biosynthesis by the YUCCA flavin monooxygenases controls the formation of floral organs and vascular tissues in Arabidopsis. *Genes Dev.* **2006**, *20*, 1790–1799. [[CrossRef](#)]
77. Kim, J.I.; Sharkhuu, A.; Jin, J.B.; Li, P.; Jeong, J.C.; Baek, D.; Lee, S.Y.; Blakeslee, J.J.; Murphy, A.S.; Bohnert, H.J.; et al. yucca6, a dominant mutation in Arabidopsis, affects auxin accumulation and auxin-related phenotypes. *Plant Physiol.* **2007**, *145*, 722–735. [[CrossRef](#)] [[PubMed](#)]
78. Zhao, Y.; Christensen, S.K.; Fankhauser, C.; Cashman, J.R.; Cohen, J.D.; Weigel, D.; Chory, J. A role for flavin monooxygenase-like enzymes in auxin biosynthesis. *Science* **2001**, *291*, 306–309. [[CrossRef](#)]
79. Chen, Q.; Dai, X.; De-Paoli, H.; Cheng, Y.; Takebayashi, Y.; Kasahara, H.; Kamiya, Y.; Zhao, Y. Auxin overproduction in shoots cannot rescue auxin deficiencies in Arabidopsis roots. *Plant Cell Physiol.* **2014**, *55*, 1072–1079. [[CrossRef](#)]
80. Cheng, Y.; Dai, X.; Zhao, Y. Auxin synthesized by the YUCCA flavin monooxygenases is essential for embryogenesis and leaf formation in Arabidopsis. *Plant Cell* **2007**, *19*, 2430–2439. [[CrossRef](#)] [[PubMed](#)]
81. Liu, Y.; Xu, M.; Liang, N.; Zheng, Y.; Yu, Q.; Wu, S. Symplastic communication spatially directs local auxin biosynthesis to maintain root stem cell niche in Arabidopsis. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 4005–4010. [[CrossRef](#)] [[PubMed](#)]
82. Brumos, J.; Robles, L.M.; Yun, J.; Vu, T.C.; Jackson, S.; Alonso, J.M.; Stepanova, A.N. Local Auxin Biosynthesis Is a Key Regulator of Plant Development. *Dev. Cell* **2018**, *47*, 306–318.e5. [[CrossRef](#)]
83. Della Rovere, F.; Fattorini, L.; D'Angeli, S.; Velocchia, A.; Falasca, G.; Altamura, M.M. Auxin and cytokinin control formation of the quiescent centre in the adventitious root apex of Arabidopsis. *Ann. Bot.* **2013**, *112*, 1395–1407. [[CrossRef](#)] [[PubMed](#)]
84. Stepanova, A.N.; Hoyt, J.M.; Hamilton, A.A.; Alonso, J.M. A Link between ethylene and auxin uncovered by the characterization of two root-specific ethylene-insensitive mutants in Arabidopsis. *Plant Cell* **2005**, *17*, 2230–2242. [[CrossRef](#)] [[PubMed](#)]
85. Moubayidin, L.; Di Mambro, R.; Sozzani, R.; Pacifici, E.; Salvi, E.; Terpstra, I.; Bao, D.; van Dijken, A.; Dello Ioio, R.; Perilli, S.; et al. Spatial coordination between stem cell activity and cell differentiation in the root meristem. *Dev. Cell* **2013**, *26*, 405–415. [[CrossRef](#)] [[PubMed](#)]
86. Di, D.W.; Wu, L.; Luo, P.; Zhang, L.; Zhang, T.Z.; Sun, X.; Wei, S.D.; An, C.W.; Guo, G.Q. Analysis the role of Arabidopsis CKRC6/ASA1 in auxin and cytokinin biosynthesis. *J. Plant Biol.* **2016**, *59*, 162–171. [[CrossRef](#)]
87. Ljung, K.; Hull, A.K.; Celenza, J.; Yamada, M.; Estelle, M.; Normanly, J.; Sandberg, G. Sites and regulation of auxin biosynthesis in Arabidopsis roots. *Plant Cell* **2005**, *17*, 1090–1104. [[CrossRef](#)]
88. Zhao, Y.; Hull, A.K.; Gupta, N.R.; Goss, K.A.; Alonso, J.; Ecker, J.R.; Normanly, J.; Chory, J.; Celenza, J.L. Trp-dependent auxin biosynthesis in Arabidopsis: Involvement of cytochrome P450s CYP79B2 and CYP79B3. *Genes Dev.* **2002**, *16*, 3100–3112. [[CrossRef](#)]
89. Jones, B.; Ljung, K. Auxin and cytokinin regulate each other's levels via a metabolic feedback loop. *Plant Signal. Behav.* **2011**, *6*, 901–904. [[CrossRef](#)]
90. Xie, M.; Chen, H.; Huang, L.; O'Neil, R.C.; Shokhirev, M.N.; Ecker, J.R. A B-ARR-mediated cytokinin transcriptional network directs hormone cross-regulation and shoot development. *Nat. Commun.* **2018**, *9*, 1604. [[CrossRef](#)]
91. Zhang, L.; Luo, P.; Bai, J.; Wu, L.; Di, D.W.; Liu, H.Q.; Li, J.J.; Liu, Y.L.; Khaskheli, A.J.; Zhao, C.M.; et al. Function of histone H2B monoubiquitination in transcriptional regulation of auxin biosynthesis in Arabidopsis. *Commun Biol.* **2021**, *4*, 206. [[CrossRef](#)]
92. Zhao, Y. Auxin biosynthesis and its role in plant development. *Annu. Rev. Plant Biol.* **2010**, *61*, 49–64. [[CrossRef](#)] [[PubMed](#)]
93. Olatunji, D.; Geelen, D.; Verstraeten, I. Control of Endogenous Auxin Levels in Plant Root Development. *Int. J. Mol. Sci.* **2017**, *18*, 2587. [[CrossRef](#)] [[PubMed](#)]
94. Lv, B.; Yan, Z.; Tian, H.; Zhang, X.; Ding, Z. Local Auxin Biosynthesis Mediates Plant Growth and Development. *Trends Plant Sci.* **2019**, *24*, 6–9. [[CrossRef](#)]
95. Mellor, N.; Band, L.R.; Pencik, A.; Novak, O.; Rashed, A.; Holman, T.; Wilson, M.H.; Voss, U.; Bishopp, A.; King, J.R.; et al. Dynamic regulation of auxin oxidase and conjugating enzymes AtDAO1 and GH3 modulates auxin homeostasis. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 11022–11027. [[CrossRef](#)]
96. Staswick, P.E.; Serban, B.; Rowe, M.; Tiryaki, I.; Maldonado, M.T.; Maldonado, M.C.; Suza, W. Characterization of an Arabidopsis enzyme family that conjugates amino acids to indole-3-acetic acid. *Plant Cell* **2005**, *17*, 616–627. [[CrossRef](#)]
97. Chen, Q.; Westfall, C.S.; Hicks, L.M.; Wang, S.; Jez, J.M. Kinetic basis for the conjugation of auxin by a GH3 family indole-acetic acid-amido synthetase. *J. Biol. Chem.* **2010**, *285*, 29780–29786. [[CrossRef](#)] [[PubMed](#)]
98. LeClere, S.; Tellez, R.; Rampey, R.A.; Matsuda, S.P.; Bartel, B. Characterization of a family of IAA-amino acid conjugate hydrolases from Arabidopsis. *J. Biol. Chem.* **2002**, *277*, 20446–20452. [[CrossRef](#)]
99. Rampey, R.A.; LeClere, S.; Kowalczyk, M.; Ljung, K.; Sandberg, G.; Bartel, B. A family of auxin-conjugate hydrolases that contributes to free indole-3-acetic acid levels during Arabidopsis germination. *Plant Physiol.* **2004**, *135*, 978–988. [[CrossRef](#)]
100. Kai, K.; Horita, J.; Wakasa, K.; Miyagawa, H. Three oxidative metabolites of indole-3-acetic acid from Arabidopsis thaliana. *Phytochemistry* **2007**, *68*, 1651–1663. [[CrossRef](#)]
101. Ostin, A.; Kowalczyk, M.; Bhalerao, R.P.; Sandberg, G. Metabolism of indole-3-acetic acid in Arabidopsis. *Plant Physiol.* **1998**, *118*, 285–296. [[CrossRef](#)]



102. Pencik, A.; Simonovik, B.; Petersson, S.V.; Henykova, E.; Simon, S.; Greenham, K.; Zhang, Y.; Kowalczyk, M.; Estelle, M.; Zazimalova, E.; et al. Regulation of auxin homeostasis and gradients in Arabidopsis roots through the formation of the indole-3-acetic acid catabolite 2-oxindole-3-acetic acid. *Plant Cell* **2013**, *25*, 3858–3870. [[CrossRef](#)] [[PubMed](#)]
103. Ljung, K. Auxin metabolism and homeostasis during plant development. *Development* **2013**, *140*, 943–950. [[CrossRef](#)] [[PubMed](#)]
104. Park, J.E.; Park, J.Y.; Kim, Y.S.; Staswick, P.E.; Jeon, J.; Yun, J.; Kim, S.Y.; Kim, J.; Lee, Y.H.; Park, C.M. GH3-mediated auxin homeostasis links growth regulation with stress adaptation response in Arabidopsis. *J. Biol. Chem.* **2007**, *282*, 10036–10046. [[CrossRef](#)] [[PubMed](#)]
105. Westfall, C.S.; Sherp, A.M.; Zubieta, C.; Alvarez, S.; Schraft, E.; Marcellin, R.; Ramirez, L.; Jez, J.M. Arabidopsis thaliana GH3.5 acyl acid amido synthetase mediates metabolic crosstalk in auxin and salicylic acid homeostasis. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 13917–13922. [[CrossRef](#)]
106. Skalicky, V.; Kubes, M.; Napier, R.; Novak, O. Auxins and Cytokinins-The Role of Subcellular Organization on Homeostasis. *Int. J. Mol. Sci.* **2018**, *19*, 3115. [[CrossRef](#)]
107. Dolan, L.; Janmaat, K.; Willemsen, V.; Linstead, P.; Poethig, S.; Roberts, K.; Scheres, B. Cellular organisation of the Arabidopsis thaliana root. *Development* **1993**, *119*, 71–84.
108. Blancaflor, E.B.; Fasano, J.M.; Gilroy, S. Laser ablation of root cap cells: Implications for models of graviperception. *Adv. Space Res.* **1999**, *24*, 731–738. [[CrossRef](#)]
109. Tsugeki, R.; Fedoroff, N.V. Genetic ablation of root cap cells in Arabidopsis. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 12941–12946. [[CrossRef](#)]
110. Swarup, R.; Kramer, E.M.; Perry, P.; Knox, K.; Leyser, H.M.; Haseloff, J.; Beemster, G.T.; Bhalarao, R.; Bennett, M.J. Root gravitropism requires lateral root cap and epidermal cells for transport and response to a mobile auxin signal. *Nat. Cell Biol.* **2005**, *7*, 1057–1065. [[CrossRef](#)]
111. Peret, B.; Swarup, K.; Ferguson, A.; Seth, M.; Yang, Y.; Dhondt, S.; James, N.; Casimiro, I.; Perry, P.; Syed, A.; et al. AUX/LAX genes encode a family of auxin influx transporters that perform distinct functions during Arabidopsis development. *Plant Cell* **2012**, *24*, 2874–2885. [[CrossRef](#)]
112. Swarup, K.; Benkova, E.; Swarup, R.; Casimiro, I.; Peret, B.; Yang, Y.; Parry, G.; Nielsen, E.; De Smet, I.; Vanneste, S.; et al. The auxin influx carrier LAX3 promotes lateral root emergence. *Nat. Cell Biol.* **2008**, *10*, 946–954. [[CrossRef](#)]
113. Swarup, R.; Friml, J.; Marchant, A.; Ljung, K.; Sandberg, G.; Palme, K.; Bennett, M. Localization of the auxin permease AUX1 suggests two functionally distinct hormone transport pathways operate in the Arabidopsis root apex. *Genes Dev.* **2001**, *15*, 2648–2653. [[CrossRef](#)]
114. Swarup, R.; Peret, B. AUX/LAX family of auxin influx carriers—an overview. *Front. Plant Sci.* **2012**, *3*, 225. [[CrossRef](#)]
115. Swarup, R.; Bhosale, R. Developmental Roles of AUX1/LAX Auxin Influx Carriers in Plants. *Front. Plant Sci.* **2019**, *10*, 1306. [[CrossRef](#)] [[PubMed](#)]
116. Adamowski, M.; Friml, J. PIN-dependent auxin transport: Action, regulation, and evolution. *Plant Cell* **2015**, *27*, 20–32. [[CrossRef](#)] [[PubMed](#)]
117. Krecek, P.; Skupa, P.; Libus, J.; Naramoto, S.; Tejos, R.; Friml, J.; Zazimalova, E. The PIN-FORMED (PIN) protein family of auxin transporters. *Genome Biol.* **2009**, *10*, 249. [[CrossRef](#)] [[PubMed](#)]
118. Zazimalova, E.; Murphy, A.S.; Yang, H.; Hoyerova, K.; Hosek, P. Auxin transporters—Why so many? *Cold Spring Harb. Perspect. Biol.* **2010**, *2*, a001552. [[CrossRef](#)] [[PubMed](#)]
119. Zwiewka, M.; Bilanovicova, V.; Seifu, Y.W.; Nodzynski, T. The Nuts and Bolts of PIN Auxin Efflux Carriers. *Front. Plant Sci.* **2019**, *10*, 985. [[CrossRef](#)]
120. Sauer, M.; Kleine-Vehn, J. PIN-FORMED and PIN-LIKES auxin transport facilitators. *Development* **2019**, *146*, dev168088. [[CrossRef](#)]
121. Cho, M.; Cho, H.T. The function of ABCB transporters in auxin transport. *Plant Signal. Behav.* **2013**, *8*, e22990. [[CrossRef](#)]
122. Verrier, P.J.; Bird, D.; Burla, B.; Dassa, E.; Forestier, C.; Geisler, M.; Klein, M.; Kolukisaoglu, U.; Lee, Y.; Martinoia, E.; et al. Plant ABC proteins—a unified nomenclature and updated inventory. *Trends Plant Sci.* **2008**, *13*, 151–159. [[CrossRef](#)]
123. Benkova, E.; Michniewicz, M.; Sauer, M.; Teichmann, T.; Seifertova, D.; Jurgens, G.; Friml, J. Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* **2003**, *115*, 591–602. [[CrossRef](#)]
124. Blakeslee, J.J.; Peer, W.A.; Murphy, A.S. Auxin transport. *Curr. Opin Plant Biol.* **2005**, *8*, 494–500. [[CrossRef](#)]
125. Friml, J.; Vieten, A.; Sauer, M.; Weijers, D.; Schwarz, H.; Hamann, T.; Offringa, R.; Jurgens, G. Efflux-dependent auxin gradients establish the apical-basal axis of Arabidopsis. *Nature* **2003**, *426*, 147–153. [[CrossRef](#)]
126. Grebe, M.; Friml, J.; Swarup, R.; Ljung, K.; Sandberg, G.; Terlou, M.; Palme, K.; Bennett, M.J.; Scheres, B. Cell polarity signaling in Arabidopsis involves a BFA-sensitive auxin influx pathway. *Curr. Biol.* **2002**, *12*, 329–334. [[CrossRef](#)]
127. Singh, G.; Retzer, K.; Vosolsobe, S.; Napier, R. Advances in Understanding the Mechanism of Action of the Auxin Permease AUX1. *Int. J. Mol. Sci.* **2018**, *19*, 3391. [[CrossRef](#)] [[PubMed](#)]
128. Kubes, M.; Yang, H.; Richter, G.L.; Cheng, Y.; Mlodzinska, E.; Wang, X.; Blakeslee, J.J.; Carraro, N.; Petrasek, J.; Zazimalova, E.; et al. The Arabidopsis concentration-dependent influx/efflux transporter ABCB4 regulates cellular auxin levels in the root epidermis. *Plant J.* **2012**, *69*, 640–654. [[CrossRef](#)]
129. Yang, H.; Murphy, A.S. Functional expression and characterization of Arabidopsis ABCB, AUX 1 and PIN auxin transporters in Schizosaccharomyces pombe. *Plant J.* **2009**, *59*, 179–191. [[CrossRef](#)]

130. Bishopp, A.; Lehesranta, S.; Vaten, A.; Help, H.; El-Showk, S.; Scheres, B.; Helariutta, K.; Mahonen, A.P.; Sakakibara, H.; Helariutta, Y. Phloem-transported cytokinin regulates polar auxin transport and maintains vascular pattern in the root meristem. *Curr. Biol.* **2011**, *21*, 927–932. [[CrossRef](#)]
131. Pernisova, M.; Prat, T.; Grones, P.; Harustiakova, D.; Matonohova, M.; Spichal, L.; Nodzynski, T.; Friml, J.; Hejatko, J. Cytokinins influence root gravitropism via differential regulation of auxin transporter expression and localization in Arabidopsis. *New Phytol.* **2016**, *212*, 497–509. [[CrossRef](#)]
132. Hansen, M.; Chae, H.S.; Kieber, J.J. Regulation of ACS protein stability by cytokinin and brassinosteroid. *Plant J.* **2009**, *57*, 606–614. [[CrossRef](#)]
133. Ruzicka, K.; Ljung, K.; Vanneste, S.; Podhorska, R.; Beeckman, T.; Friml, J.; Benkova, E. Ethylene regulates root growth through effects on auxin biosynthesis and transport-dependent auxin distribution. *Plant Cell* **2007**, *19*, 2197–2212. [[CrossRef](#)]
134. Abdollahi Sisi, N.; Ruzicka, K. ER-Localized PIN Carriers: Regulators of Intracellular Auxin Homeostasis. *Plants* **2020**, *9*, 1527. [[CrossRef](#)]
135. Petrasek, J.; Mravec, J.; Bouchard, R.; Blakeslee, J.J.; Abas, M.; Seifertova, D.; Wisniewska, J.; Tadele, Z.; Kubes, M.; Covanova, M.; et al. PIN proteins perform a rate-limiting function in cellular auxin efflux. *Science* **2006**, *312*, 914–918. [[CrossRef](#)] [[PubMed](#)]
136. Pernisova, M.; Klima, P.; Horak, J.; Valkova, M.; Malbeck, J.; Soucek, P.; Reichman, P.; Hoyerova, K.; Dubova, J.; Friml, J.; et al. Cytokinins modulate auxin-induced organogenesis in plants via regulation of the auxin efflux. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 3609–3614. [[CrossRef](#)]
137. Simaskova, M.; O'Brien, J.A.; Khan, M.; Van Noorden, G.; Otvos, K.; Vieten, A.; De Clercq, I.; Van Haperen, J.M.A.; Cuesta, C.; Hoyerova, K.; et al. Cytokinin response factors regulate PIN-FORMED auxin transporters. *Nat. Commun.* **2015**, *6*, 8717. [[CrossRef](#)] [[PubMed](#)]
138. Taniguchi, M.; Sasaki, N.; Tsuge, T.; Aoyama, T.; Oka, A. ARR1 directly activates cytokinin response genes that encode proteins with diverse regulatory functions. *Plant Cell Physiol.* **2007**, *48*, 263–277. [[CrossRef](#)] [[PubMed](#)]
139. Reyes-Olalde, J.I.; Zuniga-Mayo, V.M.; Serwatowska, J.; Chavez Montes, R.A.; Lozano-Sotomayor, P.; Herrera-Ubaldo, H.; Gonzalez-Aguilera, K.L.; Ballester, P.; Ripoll, J.J.; Ezquer, I.; et al. The bHLH transcription factor SPATULA enables cytokinin signaling, and both activate auxin biosynthesis and transport genes at the medial domain of the gynoecium. *PLoS Genet.* **2017**, *13*, e1006726. [[CrossRef](#)]
140. Zhang, W.; To, J.P.; Cheng, C.Y.; Schaller, G.E.; Kieber, J.J. Type-A response regulators are required for proper root apical meristem function through post-transcriptional regulation of PIN auxin efflux carriers. *Plant J.* **2011**, *68*, 1–10. [[CrossRef](#)]
141. Marhavy, P.; Bielach, A.; Abas, L.; Abuzeineh, A.; Duclercq, J.; Tanaka, H.; Parezova, M.; Petrasek, J.; Friml, J.; Kleine-Vehn, J.; et al. Cytokinin modulates endocytic trafficking of PIN1 auxin efflux carrier to control plant organogenesis. *Dev. Cell* **2011**, *21*, 796–804. [[CrossRef](#)]
142. Marhavy, P.; Duclercq, J.; Weller, B.; Feraru, E.; Bielach, A.; Offringa, R.; Friml, J.; Schwechheimer, C.; Murphy, A.; Benkova, E. Cytokinin controls polarity of PIN1-dependent auxin transport during lateral root organogenesis. *Curr. Biol.* **2014**, *24*, 1031–1037. [[CrossRef](#)] [[PubMed](#)]
143. Marchant, A.; Kargul, J.; May, S.T.; Muller, P.; Delbarre, A.; Perrot-Rechenmann, C.; Bennett, M.J. AUX1 regulates root gravitropism in Arabidopsis by facilitating auxin uptake within root apical tissues. *EMBO J.* **1999**, *18*, 2066–2073. [[CrossRef](#)] [[PubMed](#)]
144. Zhang, W.; Swarup, R.; Bennett, M.; Schaller, G.E.; Kieber, J.J. Cytokinin induces cell division in the quiescent center of the Arabidopsis root apical meristem. *Curr. Biol.* **2013**, *23*, 1979–1989. [[CrossRef](#)] [[PubMed](#)]
145. Franco-Zorrilla, J.M.; Lopez-Vidriero, I.; Carrasco, J.L.; Godoy, M.; Vera, P.; Solano, R. DNA-binding specificities of plant transcription factors and their potential to define target genes. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 2367–2372. [[CrossRef](#)]
146. Barbez, E.; Kubes, M.; Rolcik, J.; Beziat, C.; Pencik, A.; Wang, B.; Rosquete, M.R.; Zhu, J.; Dobrev, P.I.; Lee, Y.; et al. A novel putative auxin carrier family regulates intracellular auxin homeostasis in plants. *Nature* **2012**, *485*, 119–122. [[CrossRef](#)]
147. Feraru, E.; Vosolsobe, S.; Feraru, M.I.; Petrasek, J.; Kleine-Vehn, J. Evolution and Structural Diversification of PILS Putative Auxin Carriers in Plants. *Front. Plant Sci.* **2012**, *3*, 227. [[CrossRef](#)]
148. Sun, L.; Feraru, E.; Feraru, M.I.; Waidmann, S.; Wang, W.; Passaia, G.; Wang, Z.Y.; Wabnik, K.; Kleine-Vehn, J. PIN-LIKES Coordinate Brassinosteroid Signaling with Nuclear Auxin Input in Arabidopsis thaliana. *Curr. Biol.* **2020**, *30*, 1579–1588.e6. [[CrossRef](#)] [[PubMed](#)]
149. Paponov, I.A.; Teale, W.D.; Trebar, M.; Blilou, I.; Palme, K. The PIN auxin efflux facilitators: Evolutionary and functional perspectives. *Trends Plant Sci.* **2005**, *10*, 170–177. [[CrossRef](#)]
150. Simon, S.; Skupa, P.; Viaene, T.; Zwiewka, M.; Tejos, R.; Klima, P.; Carna, M.; Rolcik, J.; De Rycke, R.; Moreno, I.; et al. PIN6 auxin transporter at endoplasmic reticulum and plasma membrane mediates auxin homeostasis and organogenesis in Arabidopsis. *New Phytol.* **2016**, *211*, 65–74. [[CrossRef](#)] [[PubMed](#)]
151. Ding, Z.; Wang, B.; Moreno, I.; Duplakova, N.; Simon, S.; Carraro, N.; Reemmer, J.; Pencik, A.; Chen, X.; Tejos, R.; et al. ER-localized auxin transporter PIN8 regulates auxin homeostasis and male gametophyte development in Arabidopsis. *Nat. Commun.* **2012**, *3*, 941. [[CrossRef](#)] [[PubMed](#)]
152. Dal Bosco, C.; Dovzhenko, A.; Palme, K. Intracellular auxin transport in pollen: PIN8, PIN5 and PILS5. *Plant Signal. Behav.* **2012**, *7*, 1504–1505. [[CrossRef](#)]
153. Sawchuk, M.G.; Edgar, A.; Scarpella, E. Patterning of leaf vein networks by convergent auxin transport pathways. *PLoS Genet.* **2013**, *9*, e1003294. [[CrossRef](#)] [[PubMed](#)]

154. Mravec, J.; Skupa, P.; Bailly, A.; Hoyerova, K.; Krecek, P.; Bielach, A.; Petrasek, J.; Zhang, J.; Gaykova, V.; Stierhof, Y.D.; et al. Subcellular homeostasis of phytohormone auxin is mediated by the ER-localized PIN5 transporter. *Nature* **2009**, *459*, 1136–1140. [[CrossRef](#)]
155. Beziat, C.; Barbez, E.; Feraru, M.I.; Lucyshyn, D.; Kleine-Vehn, J. Light triggers PILS-dependent reduction in nuclear auxin signalling for growth transition. *Nat. Plants* **2017**, *3*, 17105. [[CrossRef](#)] [[PubMed](#)]
156. Feraru, E.; Feraru, M.I.; Barbez, E.; Waidmann, S.; Sun, L.; Gaidora, A.; Kleine-Vehn, J. PILS6 is a temperature-sensitive regulator of nuclear auxin input and organ growth in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 3893–3898. [[CrossRef](#)] [[PubMed](#)]
157. Ganguly, A.; Park, M.; Kesawat, M.S.; Cho, H.T. Functional Analysis of the Hydrophilic Loop in Intracellular Trafficking of *Arabidopsis* PIN-FORMED Proteins. *Plant Cell* **2014**, *26*, 1570–1585. [[CrossRef](#)]
158. Brenner, W.G.; Ramireddy, E.; Heyl, A.; Schmulling, T. Gene regulation by cytokinin in *Arabidopsis*. *Front. Plant Sci.* **2012**, *3*, 8. [[CrossRef](#)]
159. Brenner, W.G.; Schmulling, T. Transcript profiling of cytokinin action in *Arabidopsis* roots and shoots discovers largely similar but also organ-specific responses. *BMC Plant Biol.* **2012**, *12*, 112. [[CrossRef](#)]
160. Ruzicka, K.; Hejatko, J. Auxin transport and conjugation caught together. *J. Exp. Bot.* **2017**, *68*, 4409–4412. [[CrossRef](#)] [[PubMed](#)]
161. Dello Ioio, R.; Linhares, F.S.; Scacchi, E.; Casamitjana-Martinez, E.; Heidstra, R.; Costantino, P.; Sabatini, S. Cytokinins determine *Arabidopsis* root-meristem size by controlling cell differentiation. *Curr. Biol.* **2007**, *17*, 678–682. [[CrossRef](#)] [[PubMed](#)]
162. Grieneisen, V.A.; Xu, J.; Maree, A.F.; Hogeweg, P.; Scheres, B. Auxin transport is sufficient to generate a maximum and gradient guiding root growth. *Nature* **2007**, *449*, 1008–1013. [[CrossRef](#)] [[PubMed](#)]
163. Petersson, S.V.; Johansson, A.I.; Kowalczyk, M.; Makoveychuk, A.; Wang, J.Y.; Moritz, T.; Grebe, M.; Benfey, P.N.; Sandberg, G.; Ljung, K. An auxin gradient and maximum in the *Arabidopsis* root apex shown by high-resolution cell-specific analysis of IAA distribution and synthesis. *Plant Cell* **2009**, *21*, 1659–1668. [[CrossRef](#)]
164. Antoniadi, I.; Plackova, L.; Simonovik, B.; Dolezal, K.; Turnbull, C.; Ljung, K.; Novak, O. Cell-Type-Specific Cytokinin Distribution within the *Arabidopsis* Primary Root Apex. *Plant Cell* **2015**, *27*, 1955–1967. [[CrossRef](#)] [[PubMed](#)]
165. Armengot, L.; Marques-Bueno, M.M.; Jaillais, Y. Regulation of polar auxin transport by protein and lipid kinases. *J. Exp. Bot.* **2016**, *67*, 4015–4037. [[CrossRef](#)]